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A NEW METHOD FOR THE DETERMINATION OF HIDE QUALITY

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A method has been worked out for the quantitative determination of the extent of deterioration in hide quality during staling or during long storage based on the estimation of extractable hydroxyproline present in soak water. Estimation of extractable hydroxyproline gives a measure of the degradation of hide collagen or loss of leather making substance. The quality of dry salted hides and preserved untanned pelts may also be assessed by this method.

Introduction

It has been well emphasised from time to time that delay in curing is responsible for deterioration in hide quality caused by bacterial action on hide proteins. Salted hides also deteriorate in quality when stored for a long period. Various attempts have been made to find a suitable method for assessing the quality of raw stock. Chemical analysis of the hide for salt and moisture is an old practice existing in the trade. This method, although it gives an account of the non-leather making substances present in the hide, does not indicate the quality of the hide. The histological method[1] of assessing the quality of hide has been adopted by many investigators either alone or along with other chemical methods. But this yields only a qualitative measure of hide quality. Whitmore *et al.*[2] have found that extractable nitrogen in cured hides if present above 5% level shall indicate deterioration in hide quality. Somer[3] has correlated the volatile nitrogen content of heavy cured hides with tannery yield and has pointed out that a well cured hide may produce up to about 0·3% volatile nitrogen. But the limitations of the above methods have been discussed by Hauck and Lollar.[4]

Collagen is the basic leather making substance present in hides. It has therefore been considered that an estimation of hydroxyproline present in the hide shall give an account of the leather making potentiality of the hide. No correlation has, however, been found by Ornes and Roddy[5] between the chemical character of green salted hide and the physical strength of the finished leather.

Based on the action of proteolytic enzymes present in hide extract on pho-

tographic gelatin film, a method has recently been suggested[6] for estimating the delay in curing. This estimation of the delay in curing is, however, qualitative in nature. But the development of a convenient qualitative method to study the quality of raw hide and the extent of deterioration that has taken place in the hide warrants further investigation in this field.

In the present work, an attempt has been made to find out the interrelationship between the extractable hydroxyproline and the quality of fresh or salted hide and to use extractable hydroxyproline as an index for the quality of hide.

Methods

Estimation of hydroxyproline: Hide pieces were first dehaired with a razor, dehydrated with acetone, powdered, hydrolysed in a sealed tube with 6*N* HCl at a temperature of 105°C for 18 hours and then made up to a volume with distilled water. Hydroxyproline was then estimated by Neuman and Logan method[7] and expressed as percent on moisture-free hide.

To determine the hydroxyproline content of soak water an aliquot of 25 ml. of soak water was taken in a sealed tube, digested with conc. HCl and then dried over water bath. The dried hydrolysate was next dissolved in distilled water, made up to a known volume (100 ml) and filtered. Hydroxyproline was then estimated as before.

Tyrosine in soak water: Acid hydrolysed soak liquor that was used for the estimation of hydroxyproline was taken for tyrosine esimation. Extractable tyrosine present in different soak liquors obtained from salted hides was studied and determined by a colorimetric methods and expressed in terms of tyrosine as percent of total nitrogen.

Total nitrogen: Total hide nitrogen was estimated by digesting about 1 g. of hide sample with sulphuric acid and then estimating the nitrogen by Kjeldahl method.

Extractable nitrogen: Another aliquot of 25 ml. soak water was taken, digested with sulphuric acid and nitrogen was estimated by Kjeldahl method. Extractable nitrogen is expressed as percent of total nitrogen.

Experimental procedure and results

Extractable hydroxyproline in staled hides: A freshly slaughtered cattle hide was collected, washed to free from blood and manure and the adhering fat and flesh were removed. Experimental pieces were then cut out from the butt area. Hide pieces were allowed to be staled at a temperature of 28 ± 1 °C for different periods.

In one case staled hide pieces were taken in distilled water (1:5) and soaked for one hour with occasional hand shaking. The soak water was then filtered through filter cloth with thorough washing and made up to volume.

In the other case, hide pieces staled for different periods were salted with 40% salt on green weight and kept in a pile. After two weeks, the hide pieces were shaken free of excess salt and soaked separately in distilled water for 1 hour.

Table 1

EXTRACTABLE HYDROXYPROLINE AND NITROGEN IN UNSALTED STALED HIDE

Period of staling (hours)	Hydroxy-proline of staled hide (% of moisture free hide)	Extractable N (% of total N)	Extractable hydroxyproline N			Extractable collagen N (% of total N)
			(% of total N)	(% of extractable N)	(% of hide hydroxy-proline)	
Fresh	10·39	0·23	Nil	Nil	Nil	Nil
16	10·41	0·53	Nil	Nil	Nil	Nil
24	10·52	0·73	Nil	Nil	Nil	Nil
40	10·71	3·16	·017	·52	·009	·20
48	10·81	8·16	·046	·57	·024	·57
64	10·89	11·48	·069	·60	·037	·86

The soak liquors were then filtered and made up to volume.

Hydroxyproline, nitrogen and tyrosine contents of the soak liquors were estimated. The results are presented in Tables 1 and 2 and in Fig. 1.

It is apparent from the results in Tables 1 and 2 that the hydroxyproline content of the hide remains practically unaffected irrespective of the deterioration of hide during staling. An estimation of the hydroxyproline content of hide is thus unable to give a correct indication of the quality of hide.

Extractable nitrogen in fresh hide increases with the increase in staling period. Values for extractable nitrogen are found to differ between unsalted and salted hides. The values for unsalted hides are less up to 24 hours of staling but become higher as compared to values for salted hides on further staling. This

Table 2

EXTRACTABLE HYDROXYPROLINE AND NITROGEN IN SALTED STALED HIDE

Period of staling (hours)	Hydroxy-proline of staled hide (% of moisture free hide)	Extractable N (% of total N)	Extractable hydroxyproline N			Extractable collagen N (% of total N)
			(% of total N)	(% of extractable N)	(% of hide hydroxy-proline)	
Fresh	10·51	0·53	Nil	Nil	Nil	Nil
16	10·88	0·72	Nil	Nil	Nil	Nil
24	10·64	1·19	Nil	Nil	Nil	Nil
40	10·78	2·73	0·02	0·78	0·01	0·26
48	10·73	5·93	0·05	0·88	0·03	0·64
64	10·82	7·11	0·07	1·04	0·04	0·91

is probably because of the fact that considerable amounts of soluble proteins produced in highly staled hides due to bacterial action are drained away along with the brine during salting.

Results on extractable hydroxyproline point out that hydroxyproline is practically unextractable up to 24 hours of staling under the experimental conditions. With further staling, however, hydroxyproline is extracted progressively. There is only slight variation in extractable hydroxyproline content in salted and unsalted hides. Although extractable hydroxyproline nitrogen may be expressed in different ways, results expressed as percent of total nitrogen are more reliable.

The increase in tyrosine content in soak water obtained from salted hides which were initially staled for different periods is presented in Fig. 1. Tyrosine is found to be present only in traces in soak liquor from fresh hide and increases slowly up to 24 hours of staling and then increases progressively with further increase in staling. The low tyrosine content in the first few soak liquors obtained from less staled hides is probably due to the extraction of tyrosine containing proteins along with brine during salting.

Tyrosine is present mostly in globular proteins of hide and the data obtained thus point out that globular proteins are also degraded to a greater extent only after a staling period of 24 hours.

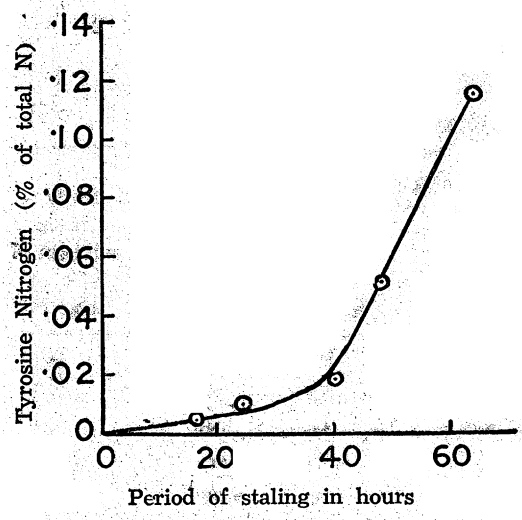

Fig. 1. Tyrosine nitrogen present in soak liquors obtained from staled salted hides

Range of variation of extractable hydroxyproline from hide samples staled for different periods

It is quite possible that extractable hydroxyproline content may vary in different areas of the hide. Extractable hydroxyproline was thus determined in different parts of the hide and after different periods of staling.

A freshly slaughtered cattle hide was taken and cut into two sides. One side was washed well with water and the other side was left unwashed. Samples were then cut from different areas of the sides e.g., butt, belly, shank and neck. These were then allowed to stale at 30 ± 1 °C for different periods. After desired saling periods, each hide piece was soaked separately and the hydroxyproline content of soak liquor was estimated as before. The ranges of variation in extractable hydroxyproline as presented in Fig. 2 are obtained from the analyses of 8 samples (4 washed and 4 unwashed) in each case.

As in previous occasions, no hydroxyproline is found to be present in soak liquors obtained from fresh and 16 hour

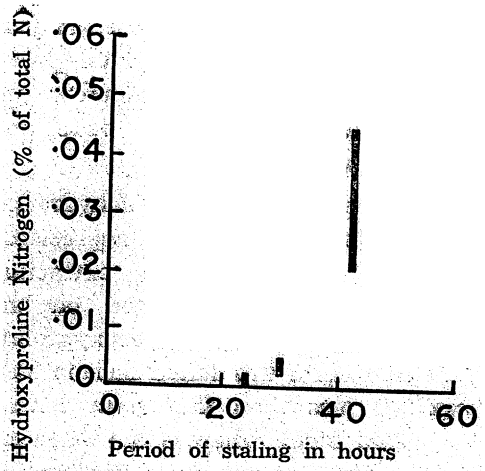

FIG. 2. Range of variation of extractable hydroxyproline nitrogen in staled hides

staled hides. Practically no hydroxyproline is found to be present in case of washed and 24 hour staled hide pieces but in unwashed hides, a small quantity of hydroxyproline is found to be extracted. Slight 'hair-slip' is also noted in unwashed hide after staling for 24 hours whereas washed hide demonstrated practically no 'hair-slip' after the same period of staling. This shows that washing of the hide may help to some extent in delaying its deterioration during staling. But as the staling period proceeds further no distinction can be made between washed and unwashed hides in respect of hydroxyproline extraction or 'hair-slip'. The range of variation is also broad after 42 hours of staling.

Effect of soaking period on extraction of hydroxyproline: The period of soaking may have a certain influence on hydroxyproline extraction and the extent of such variation was examined in the following experiment.

Fresh washed hide samples were staled for 28 hours at 30 ± 1 °C and then salted with powdered salt. Slight 'hair-slip' was notied in salted hide samples. Hide samples were then cut into small pieces (2·5 sq.inch) mixed together and divided into four lots, each weighing approximately the same. They were then soaked in distilled water in the following ways: (i) soaked for 1 hour with occasional hand shaking, (ii) soaked for 2 hours with occasional hand shaking, (iii) soaked for 1 hour in a mechanical shaker and (iv) soaked for 3 hours in a mechanical shaker. Chloroform and toluene were added to the soak liquor used for the 3 hours soaking. As before, nitrogen and hydroxyproline present in soak liquors were determined. Results are given in Table 3.

Table 3

EFFECT OF PERIOD OF SOAKING ON HYDROXYPROLINE AND NITROGEN CONTENT OF SOAK LIQUOR

Condition of soaking	Period of soaking	Extractable hydroxy-proline N	Extractable collagen N	Extractable N
		% of total N		
Shaken occasionally by hand	1 hour	004	·05	1·94
"	2 hours	004	·05	2·23
Shaken in a mechanical shaker	1 hour	004	·05	2·08
"	3 hours	004	·05	2·56

The results indicate that the period of soaking does not considerably influence the extraction of hydroxyproline from the hide. For practical purposes, soaking for a period of 1 hour in a mechanical shaker may be considered sufficient to be followed as standard practice for other experiments.

Effect of dehydration of the hide on extraction of hydroxyproline: Often the salted hides are dehydrated to different extents during storage and transport. The effect of drying of the hide on hydroxyproline extraction was thus studied.

Freshly slaughtered hide sample was staled for 42 hours at 30°C and then salted. After about 3 weeks storage, excess salt was shaken off the hide and the moisture contents was determined. This was then cut into pieces, weighed and dried in the laboratory. Moisture was determined in each case. Wet salted hide pieces were then soaked for 1 hour, partially dried pieces for 2 hours and the dry salted pieces for 3 hours, all in mechanical shaker. After soaking for the respective periods, the soak liquors were analysed for nitrogen and hydroxyproline. Data obtained in the case of partially dried and dry salted hide pieces are calculated on initial wet salted weight (39·27% moisture content) basis and presented in Table 4.

It may be observed that dehydration of the salted hide does not appreciably affect the hydroxyproline extraction (Table 4). When calculated on the initial wet salted weight, hydroxyproline extracted from a dry salted hide is only about 3% less than that from control (wet salted hide) whereas the extractable nitrogen is about 11·0% less in the case of dry salted hide. Period of soaking, however, is to be extended when dry salted hide is analysed for hydroxyproline.

Effect of primary salting of the hide with different percentages of salt on the quality of hide: The claim that the hydroxyproline extraction method can be suitably applied in assessing the quality of hide was next examined. In the first instance, the minimum quantity of salt required for primary salting was found out.

Table 4

EFFECT OF DRYING OF SALTED HIDE ON HYDROXYPROLINE AND NITROGEN CONTENT OF SOAK LIQUOR

Extent of drying	Moisture content of hide (%)	Extractable hydroxyproline N	Extractable collagen N	Extractable N
		(on initial wet salted wt. basis; % of total N)		
Wet salted	39·27	·029	·36	5·84
Partially dried	34·58	·029	·36	6·96
Dry salted	21·77	·028	·35	5·18

EXTRACTABLE HYDROXYPROLINE AND NITROGEN FROM HIDES SALTED WITH DIFFERENT PERCENTAGES OF SALT DURING PRIMARY SALTING

Percent salt applied on green weight	Condition of salted hide (observations after two weeks storage)	Extractable hydroxyproline N	Extractable collagen N	Extractable N
		% of total N		
10	Easy hair-slip, putrid smell, moist, no salt is presetnt on the surface	·009	·11	3·51
15	Slight hair-slip, slight ammoniacal smell, moist, no salt is present on the surface	·005	·06	4·74
20	No hair-slip, smell not so fresh, moist, no salt is present on the surface	Trace	Trace	1·26
25	No hair-slip, fresh smell, some salt is present on the surface	Nil	Nil	1·14

Samples were taken from freshly slaughtered cattle hide, freed from adhering fat and flesh and then salted with 10, 15, 20 and 25% salt on green weight. Salted hide pieces were taken separately in closed containers and stored at room temperature for two weeks. Observations were made on the quality of hide pieces which were then soaked for 1 hour in a shaker. Nitrogen and hydroxyproline in soak liquor were determined Results are presented in Table 5.

It is evident from the results in Table 5 that a hide salted with 20% or more salt can be preserved well for two weeks or more. Slight deterioration in hide quality has taken place within the period of observation when 15% salt is applied and considerable damage has been caused where it was salted with 10% salt.

Assessment of quality of wet salted hide during storage: The quality of salted hide as affected after different storage periods was next studied by extractable hydroxyproline method. Fresh hide samples were salted with 40% salt and then stored at room temperature (about 30°C). Samples were taken every month, soaked and the nitrogen and hydroxyproline present in soak water estimated as before. Results obtained are tabulated in Table 6.

It may be observed (Table 6) that up to 2 months' storage at a temperature of about 30°C no hydroxyproline is extracted and after 3 months, hydroxyproline is found to be extracted in traces. Hydroxyproline content in soak liquor increases with further increase in storage period. Slight 'hair-slip' is also noted after 3 months' storage when traces of hydroxyproline are found present in soak liquor.

It may further be noted that development of 'red-heat' is not always directly

Table 6

EXTRACTABLE HYDROXYPROLINE AND NITROGEN OF SALTED HIDES STORED FOR DIFFERENT PERIODS

Period of storage	Condition of salted hide	Extractable hydroxyproline N	Extractable collagen N	Extractable N
			% of total N	
1 week	Good	Nil	Nil	·56
1 month	'Red-heat' developed, otherwise good	Nil	Nil	·80
2 months	'Red-heat' developed further, otherwise same as in 1 month	Nil	Nil	1·03
3 months	'Red-heat', very slight hair-slip, smell not so fresh	Trace	Trace	1·09
4 months	Same as in 3 months but with more hair-slip	·005	·06	1·48

responsible for the degradation of collagen as is evidenced from the absence of hydroxyproline in the soak liquor even after the appearance of 'red-heat.'

Discussion

The present study reveals that good correlation exists between the extractable hydroxyproline and the quality of unsalted or salted hide. Extractable hydroxyproline is found to be absent or present in traces in hides staled up to 24 hours at a temperature of about 30°C under the existing conditions of experiment. It is thus evident that hide collagen remains practically unaffected up to 24 hours of staling after which period collagen is readily attacked by microorganisms. During the initial stages of staling the globular proteins are preferably attacked but degradation of globular proteins to a greater extent takes place possibly after 24 hours staling.

It has been observed that 'hair-slip' starts after 28-30 hours of staling at a temperature of about 30°C and becomes easy after staling for 40 hours. In some cases grain damage has been found to occur to some extent after a 40 hours staling. Thus it appears that collagen or leather making substance is not much affected till 'hair-slip' starts and an easy 'hair-slip' due to microbial action is found to be associated with loss of collagen.

It is, however, difficult to define categorically the limit of extractable hydroxyproline content that may be permitted in good quality hides but, on the basis of the present investigation, it may be said that the best quality hides should not permit any extractable hydroxyproline; hides having up to 0·005% extractable hydroxyproline nitrogen may be considered as fairly good and those containing more than 0·02% extractable hydroxyproline nitrogen as of poor quality.

Extractable nitrogen is found to vary from hide to hide and depends on other factors e.g., presence of blood and manure, type of cure etc., and so cannot be considered as a criterion for quantitative determination of hide quality. Under the present experimental conditions, it appears that extractable nitrogen for a good quality cattle hide should not be more than 3%. But this is much lower than that of the limit suggested by Whitmore *et al.*[2] and may be due to the fact that hide pieces have been soaked in their experiment for a much longer period i.e., 24 hours.

To find out the extent of deterioration that has taken place in the hide before it is dry salted, estimation of extractable hydroxyproline may provide valuable information. While extracting the hydroxyproline of dry salted hide, the soaking period should, however, be extended to 3 hours or more.

Recommended procedure

A hide piece weighing about 50 g. is cut out, cleaned free of excess salt and surface blood, taken in a bottle with 5 times its weight of distilled water and shaken in a mechanical shaker for 1 hour. The soak liquor is then filtered and made up to 250 ml. in a volumetric flask. Hydroxyproline is then estimated and calculated as hydroxyproline nitrogen and expressed as percent of total hide nitrogen. This method is quite simple and will give a quantitative measure of the hide quality.

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